

SEPT3 as a Potential Molecular Target of Triple-Negative Breast Cancer

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Background: The survival rate for triple-negative breast cancer (TNBC) is very low due to its advanced metastatic and aggressive nature, and there is no specific target to improve the survival rate. The expression and clinical signature of neuronal-specific septin-3 (Septin3, SEPT3) in TNBC remain undetermined.

Methods: SEPT3 differential expression in TNBC was detected with the use of bioinformatic approaches based on TCGA and GEO database, which was verified with immunohistochemistry in TNBC tissues. Next, the effect of SEPT3 on survival and the association between SEPT3 expression and clinical characteristics were assessed for TNBC patients. We performed Cox analysis to evaluate whether SEPT3 is an independent predictor for TNBC patients.

Results: SEPT3 was identified as a key differentially expressed gene. SEPT3 was observed to be elevated in 112 TNBC significantly. Increased expression of SEPT3 contributed to an unfavorable prognosis in patients with TNBC. Additionally, SEPT3 was associated with several factors including TNM stage, lymph node metastasis, Ki67 level and histological grade. SEPT3 was determined to be an independent risk factor for TNBC patients through Cox regression analysis.

Conclusion: This study demonstrated that SEPT3 could be a potential disease marker for TNBC patients by bioinformatics analysis and validation in clinical samples.

Keywords: triple-negative breast cancer, SEPT3, prognosis, biomarker

Introduction

As a heterogeneous disease, the absence or presence status of hormone receptors and human epidermal growth factor receptor-2 (HER2) is used to classify breast cancer subtypes.¹ Triple-negative breast cancer (TNBC) is the one that lacks detectable expression of three index: estrogen and progesterone receptors (ER, PR) and HER2. Given these properties, endocrine therapy and targeted therapy have poor efficacy for patients with TNBC.² Recently, the clinical treatment for TNBC is mainly chemotherapy combining paclitaxel and platinum with little benefit.³ Therefore, the poor prognosis of TNBC patients requires identifying specific molecular targets.

Septins, a highly conserved guanosine triphosphate (GTP) binding protein family, can be divided into four homology subgroups.⁴ In addition to their GTPase function, septins can take part in initiating homo- or heterooligomeric complexes, which are essential for maintaining the cytoskeleton.⁵ Recently, the role of septins in cancer was gradually excavated. Septin9 was found to be overexpressed in human breast cancer.⁶ In colorectal cancer, Septin9 could serve as a specific blood marker.⁷ Besides, Septin9 could affect cell invasion and metastasis in melanoma.⁸ Cancer-associated fibroblasts (CAFs) tend to facilitate cancer invasion and angiogenesis via remodeling the tumor matrix. It has been reported that Septin2 was involved in the regulation of matrix remodeling and tumor-growth promoting mediated by CAFs.⁹ A growing body of evidence demonstrated that septins play a key role in cancer.

Neuron-specific septin-3 (Septin3, SEPT3), of the SEPT3 subgroup, is present in the brain and testes. In previous studies, STEP3 was identified as a component of presynaptic nerve terminals with lipid association and GTP-regulated filament assembly properties.¹⁰ Conversely, accumulating evidence indicated that the effects of SEPT3 on neuronal development and synaptic function are negligible.^{11–13} The existing body of research on SEPT3 suggested that SEPT3 correlates with autophagy in synaptic/neuronal tissue. In primary neuronal cell cultures, SEPT3 binds the autophagy-related protein LC3B, and colocalization of these proteins can be enhanced by autophagy inducers.¹⁴ Current studies have focused on the biological effect of SEPT3 in the nervous system, while its expression pattern and prognostic value in cancer have not been reported.

The expression pattern, clinical significance and prognostic value of SEPT3 in TNBC have not been investigated. This study is the first to deeply uncover the role of SEPT3 in TNBC according to the bioinformatics analysis and clinical sample validation. Next, we explored the clinical significance of SEPT3 in TNBC. The study provided a pipeline for exploration of disease markers and prognostic risk factors in TNBC.

Methods

Bioinformatics Analysis

TCGA RNA data of breast cancer and TNBC were downloaded from the Cancer Genome Atlas website (<http://cancergenome.nih.gov>). Next, the validation cohort of TNBC was extracted from Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/; GSE76250, GSE38959). Differentially expressed genes (DEGs) were identified with the following screening conditions: |log2 Fold Change| and q-value. The Venn diagram was applied to produce the common DEGs. Next, DEGs were assigned for prognostic analysis by Log rank tests and univariate cox regression, which were presented as forest plots. We divided patients into two groups according to the expression of SEPT3. The overall survival distributions between two groups were compared via the Kaplan–Meier plotter database (www.kmplot.com). Cox regression analysis was performed univariately and multivariately to determine whether SEPT3 was a prognostic factor.

TNBC Patients' Specimens

There were 112 paired TNBC tissues and adjacent normal samples collected from patients with resection of cancer who had been treated without receiving chemotherapy beforehand at Huangshi Central Hospital. The patients provided written consent and all aspects of this study were approved by the ethical committee of Huangshi Central Hospital (NO. 2022–14). Overall survival is defined as the time from the date of surgery to the time of death.

Immunohistochemistry (IHC)

Hematoxylin and eosin (HE) staining was conducted according to routine protocols. The expression level of SEPT3 was detected by IHC with anti-STEP3 (1:500, Abcam, UK). A gradient of xylenes and ethanol was used to deparaffinize and rehydrate all sections, respectively. All sections were submerged into citrate buffer, followed by 3% hydrogen peroxide. The next step was to block nonspecific binding by using goat serum. The primary antibodies were added with sections overnight at 4 °C. For visualization, the sections were stained with diaminobenzidine after being incubated for 60 minutes with a secondary antibody. In the cohort of 112 samples, SEPT3 expression levels were categorized by a cutoff score into high- and low-expression groups.

Statistical Analysis

R v4.0.2 and SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) were used to conduct statistical analysis and graph visualization. Clinicopathologic parameters in different groups were statistically analyzed with a chi-squared test. Survival analysis was performed by the Kaplan–Meier method, and the Log rank test was used to compare the survival rates between the two groups. The Cox proportional hazards model was used to analyze the joint effect of covariates. *P*-value less than 0.05 was considered statistically significant.

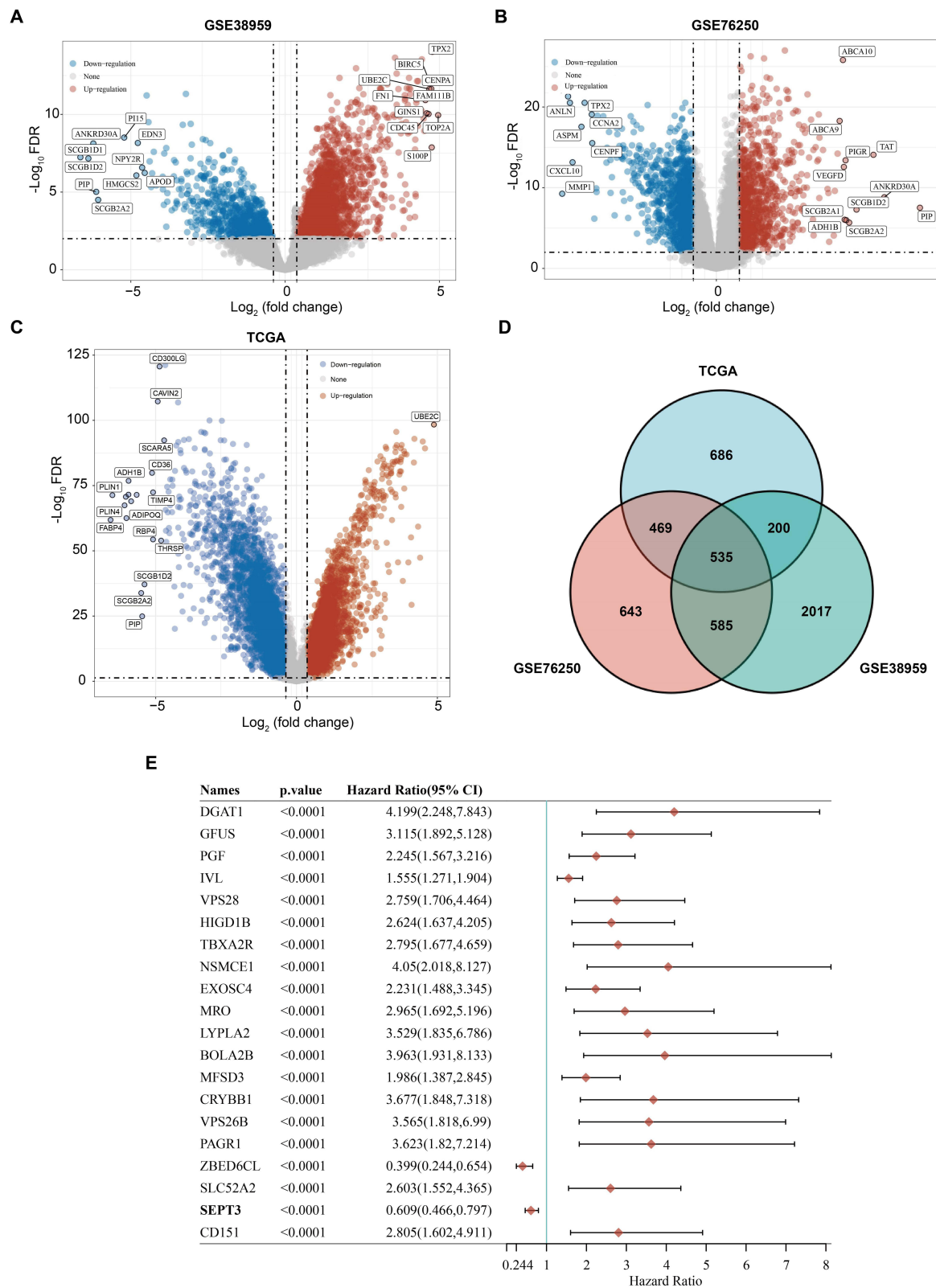


Figure 1 SEPT3 is identified as a disease marker in TNBC. (A–C) Volcano plots represent the differential genes between TNBC and normal samples based on GSE76250, GSE38959 and TCGA datasets. (D) Venn diagram demonstrates the intersections of genes among three datasets. (E) Forest plot shows that differentially expressed genes associated with TNBC prognosis, SEPT3 is closely related to the prognosis of patients with TNBC.

Results

SEPT3 is Identified as a Prognostic Marker in TNBC

According to TCGA and GEO database, DEGs between TNBC and normal tissues were presented in the form of volcano (Figure 1A–C). As shown in Figure 1D, the common candidate genes among three datasets were displayed. The above genes were prepared for the following cox regression analysis. A forest map identified that DEGs were significantly associated with TNBC prognosis (Figure 1E). Based on above selection, SEPT3 was included in the following analysis.

We next assessed the expression level of SEPT3 between TNBC and normal tissues using TCGA and GEO database. We observed a significantly increased expression level of SEPT3 in TNBC compared to normal tissues ($P < 0.001$, Figure 2A). There was a significant correlation between higher expression of SEPT3 and a worse prognosis on survival curves (Figure 2B). The model of univariate cox proportional regression suggested that SEPT3 is related to survival (Figure 2C). In Cox multivariate analysis, SEPT3 was shown to be an independent risk factor for TNBC (Figure 2D).

Characteristics of TNBC Patients from a Clinicopathological Perspective

A total of 112 TNBC cases were enrolled in this study. The patient’s clinicopathological characteristics are shown in Table 1. The majorities had a moderate histological grade (58.3%). Seventy-eight patients (69.6%) had negative lymph

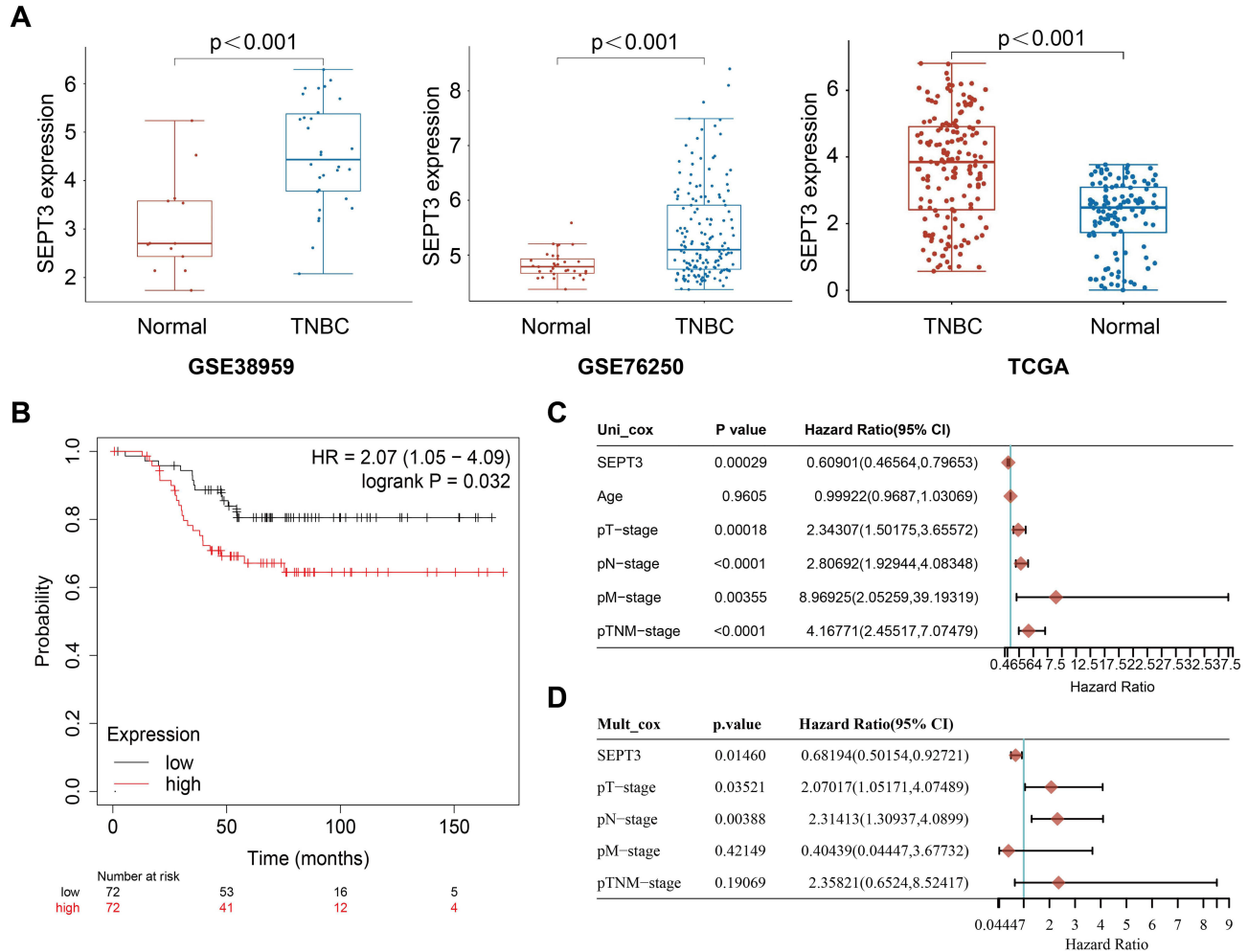


Figure 2 SEPT3 can be used as independent prognostic factor for TNBC patients. (A) Differences in the expression of SEPT3 between normal and tumor tissues based on three datasets. (B) Kaplan-Meier survival curves of TNBC patients stratified by high and low expression of SEPT3. (C) Univariate Cox regression analysis. (D) Multivariate Cox regression result.

Table 1 Association Between SEPT3 Expression and Clinical Features of Patients

Features	SEPT3 Expression			χ^2	P-value
	Total (n=112)	Low (n=52)	High(n=60)		
Age (years)				0.308	0.579
<50	57	25 (48.1)	32 (53.3)		
>50	55	27 (51.9)	28 (46.7)		
Tumor size (mm)				2.152	0.142
<20	65	34 (65.4)	31 (51.7)		
>20	47	18 (34.6)	29 (48.3)		
TNM stage				5.635	0.018
I-II	72	39 (75.0)	32 (53.3)		
III-IV	40	13 (25.0)	28 (46.7)		
LN metastasis				7.818	0.005
N0	78	43 (82.7)	35 (58.3)		
N1-N3	34	9 (17.3)	25 (41.7)		
Histological grade				6.023	0.049
G1	22	14 (26.9)	7 (11.7)		
G2	65	30 (57.7)	35 (58.3)		
G3	25	8 (15.4)	18 (30)		
Menstrual status				0.924	0.337
Postmenopausal	55	23 (44.2)	32 (53.3)		
Others	57	29 (55.8)	28 (46.7)		
Pathologic types				0.700	0.403
IDC	65	28 (53.8)	37 (61.7)		
Other	47	24 (46.2)	23 (38.3)		
Ki 67 (%)				4.435	0.035
<20	66	37 (71.2)	31 (51.7)		
>20	46	15 (28.8)	29 (48.3)		

node metastases in surgical specimen; 72 patients (64.3%) presented TNM stage (I–II). The pathologic types were predominantly of invasive ductal carcinoma (IDC).

Verification of SEPT3 Expression in TNBC Tissues

Clinical samples were analyzed for SEPT3 expression to provide further validation. In the first step, a HE staining was utilized to identify the tumor cells and tissues (Figure 3A). Next, we quantified protein expression levels of SEPT3 by IHC. As shown in Figure 3B, SEPT3 staining was intense in TNBC tissues, while more weak staining was observed in normal tissues. SEPT3 was mainly found in the cytoplasm. The high expression rate of SEPT3 in TNBC samples was significantly higher than that in normal samples, which indicates that SEPT3 exhibits a higher expression pattern in TNBC.

The association between SEPT3 and clinical pathological features was evaluated based on the IHC score. The patients were divided into two groups, one with low levels of SEPT3 expression (n=50) and the other with high levels of SEPT3 expression (n=62). As shown in Table 1, increased expression of SEPT3 positively correlated with higher TNM stage, lymph node metastasis, histological grade and Ki67 expression (P=0.018, P=0.005, P=0.049, P=0.035). A relationship between SEPT3 expression and age, tumor size, menstrual status, or pathologic types was not found. The above findings suggested that SEPT3 may contribute to the tumor malignant progression and promotion of cancer cell proliferation.

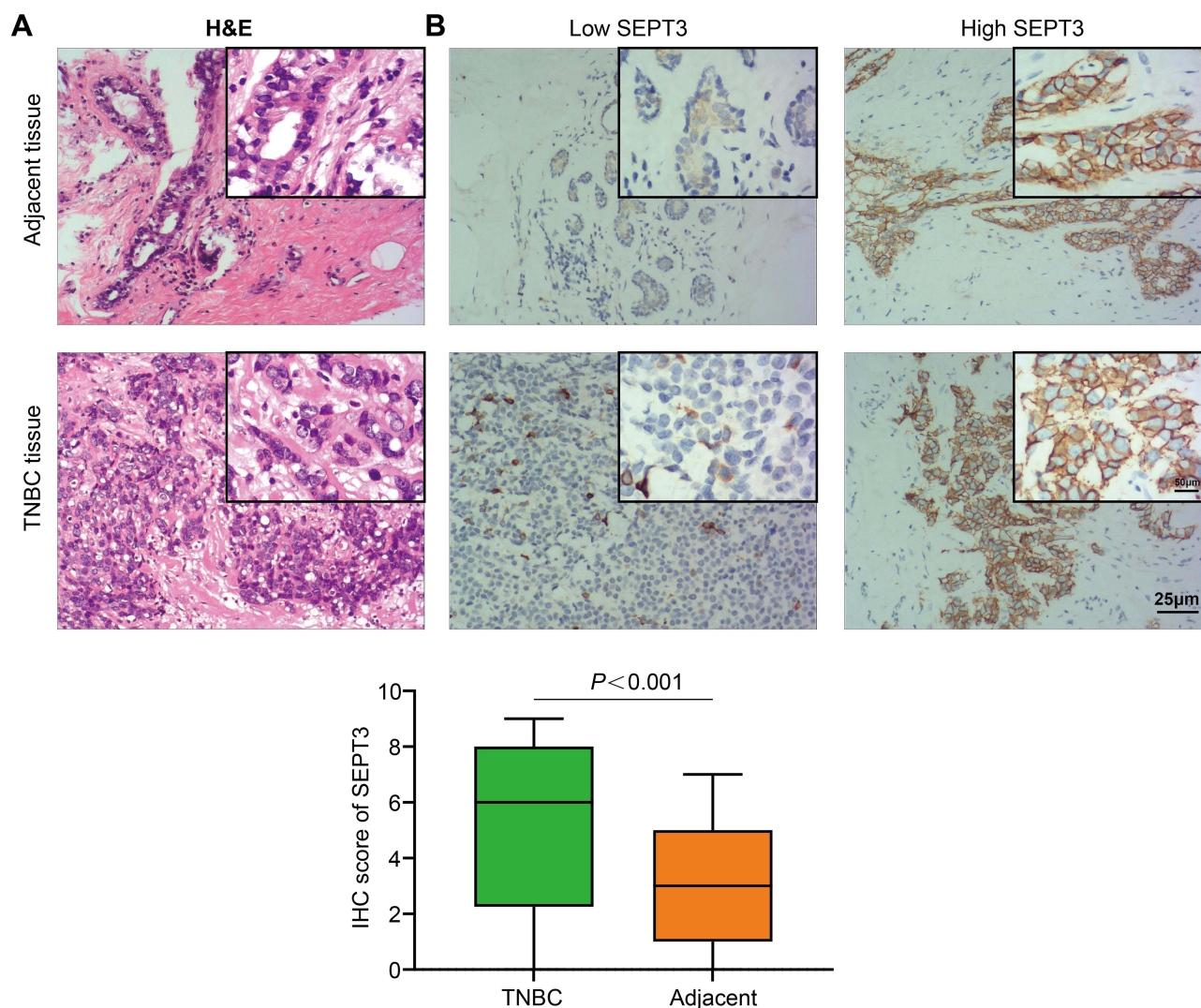


Figure 3 Validation expression value of SEPT3 in clinical specimen. (A) HE staining of TNBC tissue and the adjacent normal tissues. (B) IHC demonstrates immuno-expression of SEPT3 in TNBC tissues.

STEP3 is an Independent Risk Factor for TNBC Patients

We next assess the effect of SEPT3 expression on prognosis. Results of the Kaplan–Meier survival curve indicated that patients with high SEPT3 expression levels had a less favorable survival than those with low SEPT3 expression levels ($P=0.032$) (Figure 4A–C). Univariate and multivariate Cox regression analysis showed that SEPT3 is an independent predictor for TNBC patients after adjusting for TNM stage, lymph node metastasis and Ki67 expression level ($P=0.047$, $HR=1.212$, Figure 4D). According to these results, patients with TNBC have a worse prognosis with higher SEPT3 expression.

Discussion

TNBC remains a devastating malignancy and the number of new deaths related to TNBC is increasing.¹⁵ Due to the heterogeneity of TNBC, the demand for TNBC prognostic and therapeutic markers is intensifying. Increasing evidence supported that bioinformatics technologies is a strong tool to discover novel targets for cancer.^{16,17} Therefore, the combination of bioinformatic approaches and experimental validation was an attractive strategy for TNBC patients. Recently, most studies focused on the screening of DEGs for prognostic prediction and treatment strategy.^{18,19} In this

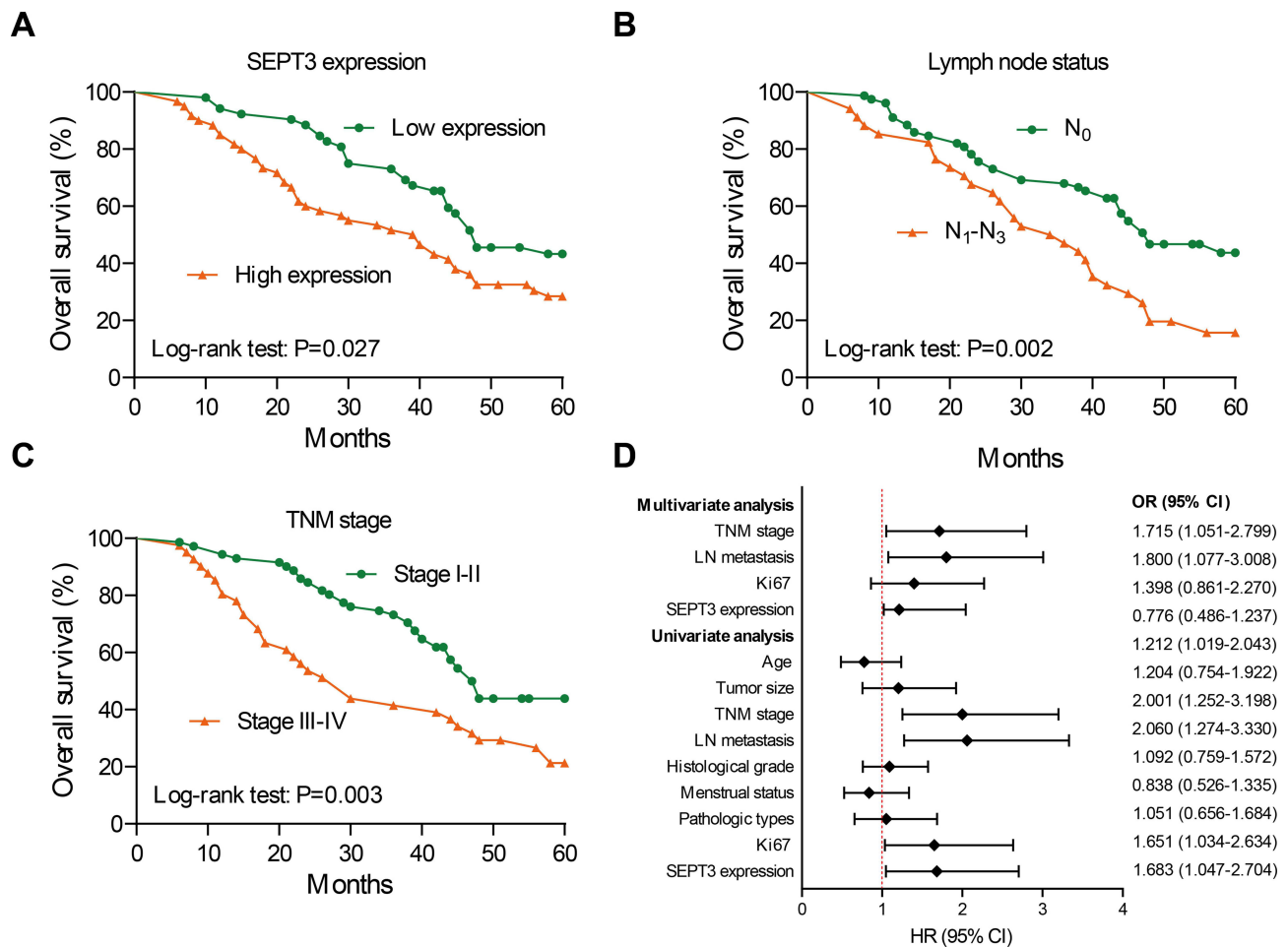


Figure 4 Survival curve of the effect of SEPT3 on the survival time of TNBC patients. (A) Overall survival according to status of SEPT3 expression. (B) Overall survival according to lymph node status. (C) Overall survival according to TNM stage. (D) Univariate and multivariate analysis for overall survival.

study, we identified SEPT3 as a potential target for TNBC patient with the cox regression analysis based on multiple data sets.

The septin-3 protein is a member of the septin protein family that forms multimers and facilitates the formation of cytoskeletons. Previous studies mainly investigated the effect of SEPT3 on neurological disorders, such as Alzheimer's disease (AD). AD is characterized by synaptic dysfunction. The accumulation of SEPTIN3 and SEPTIN5 might compromise synaptic transmission based on animal models.²⁰ Using shotgun mass spectrometry, Sravani et al reported that SEPT3 might be a potential disease marker for AD.²¹ The expression pattern and prognostic value of septin-3 has yet to be described in cancer. From the review of literature, only one report has been published. In brain tumors, SEPT3 was observed in medulloblastoma tissues and cell lines, but not in astrocytoma tissues and cell lines.²² In this study, SEPT3 was found to be elevated in TNBC tissues from both databases and TNBC specimen. SEPT3 expression contributed to an adverse prognosis in TNBC through survival analysis, suggesting SEPT3 expression is predictive of poor prognosis. Interestingly, according to the correlation analysis between SEPT3 expression and clinical features, we found that SEPT3 may affect the progression of TNBC, especially lymph node metastasis. SEPT9, the same member of septins, has been reported to be related to the tumor growth and survival. The underlying mechanism was that SEPT9 expression could inhibit epithelial-mesenchymal transition induced by TGF- β 1.²³ A recent study concluded that SEPT3 might be involved in regulation of cellular migration mediated by extracellular vesicles in mesenchymal stem cells.²⁴ Taken together, SEPT3 belongs to cytoskeleton proteins that may promote TNBC cell metastasis.

In addition to a relationship between tumor occurrence and metastasis, SEPT3 might be involved in tumor growth. The results of IHC demonstrated that SEPT3 expression was positively correlated with Ki67 expression. Septins has been reported to be a cell cycle-regulated protein, which facilitates the process of cytokinesis in human astrocytoma cells.²⁵ As a consequence of septin overexpression, cytokinesis is compromised, centrosome amplification occurs, and multipolar mitoses occur, which are characteristics of cancer cells.²⁶ However, the exact mechanism of tumor growth mediated by SEPT3 has not been discovered. The biological function and mechanisms of SEPT3 in TNBC need to be further verified.

Conclusions

Our study indicated that SEPT3 could be a potential predictor for TNBC prognosis. Patients with TNBC may benefit from targeting SEPT3. In this study, we provide a foundation for identifying and treating TNBC patients at an early stage.

Data Sharing Statement

All data can be obtained in TCG, GEO and conclusions of this article will be made available by the corresponding author.

Ethics Approval

All procedures performed were in accordance with the declaration of the ethical standards of the institutional research committee and with the 1964 Helsinki 387 Declaration and its later amendments. The ethics committee has approved this study of the Huangshi Central Hospital.

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Disclosure

All authors declare no conflicts of interest for this work.

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